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CLINICAL RESEARCH
Prevention/epidemiology

Common variants in *RYR1* are associated with left ventricular hypertrophy assessed by electrocardiogram

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Aims

To identify the genetic risk factors that influence the development of electrocardiographic (ECG) left ventricular hypertrophy (LVH), a major risk factor for cardiovascular (CV) morbidity and mortality.

Methods and results

We performed a genomewide association study (GWAS) of ECG-LVH, in which the community-based Korea Association Resource (KARE) study (8432 controls and 398 cases) was analysed by Affymetrix SNP array 5.0. The GWAS results were validated in hospital-based samples (597 controls and 207 cases). Fourteen single-nucleotide polymorphisms (SNPs) in eight genetic loci (5q35.1, 6p22.3-22.1, 8q24.2, 11p15, 11q21-22.1, 14q12, 17q11.2, and 19q13.1) were associated with ECG-LVH in the original GWAS study ($P < 1 \times 10^{-5}$). Of these SNPs, 12 were genotyped in the hospital sample. There was consistent association with the 19q13.1 region which contains *RYR1* gene. The most significant SNP in the region was rs10500279, which had genomewide significance in the combined GWAS/replication sample [odds ratio = 1.58 (confidence interval: 1.35–1.85), $P = 1.0 \times 10^{-8}$]. Mutations in *RYR1*, which encodes a major Ca^{2+} channel in the skeletal muscle, have been reported to correlate with CV diseases.

Conclusion

We performed the first GWAS for ECG-LVH, implicating the skeletal muscle Ca^{2+} channel protein *RYR1* as a genetic risk factor. These results might increase our understanding of the development of ECG-LVH.

Keywords

Genomewide association study • Left ventricular hypertrophy • Electrocardiography • Ryanodine receptor • Cardiovascular disease

Introduction

Left ventricular hypertrophy (LVH) is a major risk factor for cardiovascular (CV) morbidity and mortality. Left ventricular hypertrophy is diagnosed by electrocardiography (ECG) and echocardiography.^{1–4} Echocardiography is a more sensitive and specific method of detecting LVH than ECG, but its use in large-scale population studies and clinical trials is limited by its prohibitive cost and operational considerations.⁵

In contrast, ECG is widely available, inexpensive, and less operator-dependent⁶; thus, ECG data are obtainable in nearly all patients and participants in epidemiological studies.⁷

Left ventricular hypertrophy is a multifactorial trait; its major determinants include blood pressure (BP), age, gender, and obesity.⁸ In addition, certain genetic factors, such as angiotensin-converting enzyme,⁹ guanine nucleotide-binding protein (GNB3),¹⁰ insulin-like growth factor,¹¹ and neuropeptide Y,¹² regulate the development of LVH. Several studies have reported

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a relationship between LVH and variations in genes that are associated with the renin–angiotensin–aldosterone system^{13,14} and nitric oxide synthase.¹⁵

Recently, the EchoGen consortium ($n = 16\,706$ subjects of European ancestry, based on five community cohorts) performed a seminal genomewide association study (GWAS) on cardiac structure, based on echocardiography measurements.¹⁶ The GWAS identified one locus (6q22) that correlated with LV diastolic dimensions and four loci (5q23, 12p12, 12q14, and 17p13) that were linked to the aortic root size.¹⁶ Its results, however, explained merely 1–3% of the trait variance that was observed.¹⁶ Considering that the heritability estimate of LV mass ranges between 0.17 and 0.59, many genetic factors remain to be identified.^{17–20}

Most studies that have sought to determine the genetic influence on LVH have used echocardiography to measure LV mass. Yet, a family study by Mayosi *et al.*^{7,21} estimated higher heritability rates for LVH detected by ECG (ECG-LVH) (39–41%) compared with LVH determined by echocardiography (ECHO-LVH) (21–29%), suggesting that there may be greater genetic susceptibility for ECG-LVH.

Here, we report the first GWAS on ECG-LVH using population-based community cohorts and replicate the results in hospital-based sample to identify genetic risk factors that influence the development of ECG-LVH.

Methods

Original study subjects

The Korea Association REsource (KARE) subjects were described previously.²² Briefly, the subjects came from two community-based cohorts—the rural Ansung community and the urban Ansan community—both located in KyungGi-Do province near Seoul, Korea. Initially, there were 5018 and 5020 individuals, aged 40–70 years, from Ansung and Ansan, respectively.

Subjects with genotype accuracies below 98% and high missing genotype call rates ($\geq 4\%$), high heterozygosity ($> 30\%$), or inconsistency in sex were excluded from subsequent analyses. Individuals who had a tumour were excluded, as were related individuals whose estimated identity-by-state values were high (> 0.80). After these quality control steps, 8842 samples were selected, of whom 12 did not undergo ECG. Ultimately, 8830 samples were used for the GWAS.

Left ventricular hypertrophy was diagnosed by ECG, based on the Minnesota Code Classification System,²³ if R amplitude is > 26 mm in V5 or V6; R amplitude > 20 mm in leads I, II, or III; or aVF or R amplitude > 12 mm in the lead aVL (all criteria were measured on the penultimate complete normal beat).

Blood pressure was measured three times in the supine position, and the average value was used for the GWAS. Before the first measurement, participants rested for 5 min, and the three measurements were taken in one arm showing the higher BP at least 3 min apart. Other CV risk factors, such as cholesterol level and fasting glucose level, were measured from blood samples after overnight fasting.

Original study genotypes

Most DNA samples were isolated from the peripheral blood of participants and genotyped using the Affymetrix Genomewide Human SNP array 5.0 (Affymetrix, Inc., Santa Clara, CA, USA). The quality control steps of genotypes have been described elsewhere.²²

Briefly, the accuracy of the genotyping was determined by Bayesian Robust Linear Modeling using the Mahalanobis Distance (BRLMM) genotyping algorithm.²⁴ Consequently, 333 651 single-nucleotide polymorphisms (SNPs) had a missing genotype call rate below 0.1, a minor allele frequency (MAF) > 0.01 , and no deviation from the Hardy–Weinberg equilibrium (HWE) ($P > 1 \times 10^{-6}$). To examine the population stratification, multidimensional scaling analysis and principal component analysis were performed using pruned 44 724 SNP markers among all KARE.²²

Replication study subjects

The replication study included 207 LVH patients and 597 normal controls, selected from the patient database at the Cardiovascular Genome Center, Yonsei University Health System, Seoul, Korea. Both case and control subjects were independent from those of the original GWAS study. The patient database comprises individuals aged 40–70 years who entered the outpatient clinic or were hospitalized between May 2002 and November 2007 in the Cardiology Division of Severance Cardiovascular Hospital. Electrocardiographic-left ventricular hypertrophy was diagnosed using the same criteria as in the original GWAS—the Minnesota Code Classification System.²³ The local Ethics Committee approved this study, and informed consent was obtained from all patients.

Replication study genotypes

Single-nucleotide polymorphism selection for the replication study was based on the significance with P -value ($< 1 \times 10^{-5}$). The SNPs were genotyped by TaqManTM fluorogenic 5' nuclease assay (Applied Biosystems, Foster City, CA, USA). The final volume of each polymerase chain reaction (PCR) was 5 μ L, containing 5 ng genomic DNA, 2.5 μ L TaqManTM Universal PCR Master Mix, and 0.13 μ L 40 \times pre-designed TaqMan probe Assay Mix.

The thermal cycling programme was as follows: 2 min at 50°C to activate uracil N-glycosylase and prevent contamination, 10 min at 95°C to activate the DNA polymerase, and 45 cycles of 15 s at 95°C and 1 min at 60°C. All reactions were run in 384-well plates on a Dual 384-Well GeneAmp[®] PCR System 9700 (Applied Biosystems) and read on an ABI PRISM 7900 HT Sequence Detection System (Applied Biosystems). Duplicate samples and negative controls were included to ensure the accuracy of the genotyping data.

Statistical analysis

The ECG-LVH cases and controls were analysed by logistic regression, controlling for covariates, such as antihypertensive drug treatment state, cohort, age, sex, body mass index (BMI), systolic BP (SBP), diastolic BP (DBP), and HDL, LDL, triglyceride, and fasting glucose levels. Statistical analyses were performed using PLINK, version 1.07, using default options²⁵ and SPSS, v15.0. For the multicollinearity of covariates, we estimated the tolerance and variance inflation factor (VIF) by using the SPSS and described the results in Table 1. Multiple testing of association results was conducted by the Bonferroni correction criteria. The asymptotic HWE tests were conducted using PLINK, and all the reported P -values were two-sided.

Results

Baseline characteristics of subjects

Three hundred and ninety-eight individuals out of the 8830 community-based KARE subjects were diagnosed as ECG-LVH by the Minnesota Code Classification System. The demographics

Table 1 Clinical characteristics of the KARE and replication study subjects

	KARE subjects		Case vs. control, <i>P</i> -value ^a	Collinearity statistics	Replication study subjects		Case vs. control, <i>P</i> -value ^a	Collinearity statistics		
	Control	Case			Tolerance	VIF			Control	Case
<i>n</i>	8432	398				597	207			
Treated ^b (%)	1217 (14)	72 (18)	4.3 × 10 ^{−2}	0.90	1.11	99 (17)	194 (95)	4.8 × 10 ^{−43}	0.77	1.30
Men (%)	3916 (46)	261 (66)	3.0 × 10 ^{−8}	0.90	1.11	430 (72)	161 (78)	2.5 × 10 ^{−1}	0.89	1.12
	Mean (SD)					Mean (SD)				
Age	52.1 (8.9)	55.1 (9.2)	2.2 × 10 ^{−11}	0.81	1.24	57.4 (7.5)	58.3 (8.0)	1.2 × 10 ^{−2}	0.88	1.14
Body mass index	24.6 (3.1)	23.6 (2.9)	1.1 × 10 ^{−11}	0.80	1.24	24.2 (2.6)	24.5 (3.4)	3.0 × 10 ^{−1}	0.92	1.09
Systolic blood pressure (mmHg)	117.1 (18.0)	128.0 (20.8)	1.7 × 10 ^{−31}	0.29	3.41	124.3 (17.9)	126.9 (21.0)	2.7 × 10 ^{−1}	0.42	2.35
Diastolic blood pressure (mmHg)	74.9 (11.5)	79.4 (11.7)	1.6 × 10 ^{−14}	0.32	3.08	78.4 (10.9)	77.6 (11.2)	2.6 × 10 ^{−1}	0.43	2.34
HDL cholesterol (mg/dL)	44.6 (10.1)	45.3 (10.6)	1.7 × 10 ^{−1}	0.80	1.25	48.9 (15.2)	44.0 (12.9)	9.7 × 10 ^{−9}	0.90	1.11
LDL cholesterol (mg/dL)	116.0 (32.2)	110.3 (32.7)	6.8 × 10 ^{−4}	0.93	1.07	120.1 (35.3)	97.0 (33.7)	1.2 × 10 ^{−2}	0.87	1.15
Triglyceride (mg/dL)	163.0 (106.1)	162.6 (98.6)	9.4 × 10 ^{−1}	0.77	1.30	140.8 (95.0)	135.1 (103.0)	5.6 × 10 ^{−1}	0.91	1.10
Fasting glucose level (mg/dL)	87.7 (22.0)	86.9 (19.4)	4.8 × 10 ^{−1}	0.94	1.06	96.1 (27.4)	114.1 (52.1)	1.6 × 10 ^{−6}	0.84	1.20

SD, standard deviation; VIF, variance inflation factor.

^a*P*-values between control and case were calculated using χ^2 for treated and sex ratio, and Student's *t*-test for quantitative traits.

^bAntihypertensive drug-treated subjects.

and clinical characteristics of the subjects are shown in Table 1. Antihypertensive treatment status, sex, age, BMI, SBP, DBP, and LDL levels differed significantly between the case and control groups of the KARE GWAS. Of the controls in the GWAS, 1217 (14%) subjects had taken antihypertensive medications compared with 72 (17%) cases.

In the hospital-based replication study, antihypertensive treatment status, age, HDL, LDL, and fasting glucose levels differed significantly between the cases and controls. Ninety-nine (17%) of controls had taken antihypertensive medications, as did 194 (95%) cases.

To avoid the multicollinearity of covariates, we tested the tolerance and VIF of them. The test results showed all VIF values lower than 5, the suggested conservative threshold, suggesting the low multicollinearities of covariates.²⁶

Electrocardiographic-left ventricular hypertrophy genomewide association study

The ECG-LVH GWAS used KARE genotyped data previously reported by Cho et al.²² A GWAS of 333 651 SNPs was performed using community-based LVH cases (*n* = 398) and controls (*n* = 8432); a quantile–quantile (Q–Q) plot is shown in Supplementary material online, Figure S1A. The genomic inflation factor (λ) was 1.00, which was evidence against population stratification or inflated results.

Figure 1A illustrates the Manhattan plot of the GWAS results; the results that are based on *P*-values lower than 1 × 10^{−5} are summarized in Table 2. None of the *P*-values for the associations met the multiple comparison criteria (Bonferroni's correction

P < 1.5 × 10^{−7}). Therefore, instead of Bonferroni's correction criteria, a less stringent *P*-value (<1 × 10^{−5}) was applied to select suggestive signals for further study in the replication sample.

The GWAS on ECG-LVH identified 14 SNPs in eight suggestive association loci: one SNP in 5q35.1 (rs265992, *P* = 1.2 × 10^{−6}), one SNP in 6p22.3-22.1 (rs9295629, *P* = 9.0 × 10^{−6}), five SNPs in 8q24.2 (the most significant SNP: rs4909705, *P* = 3.7 × 10^{−6}), one SNP in 11p15 (rs17446021, *P* = 4.9 × 10^{−6}), one SNP in 11q21-q22.1 (rs11225822, *P* = 1.4 × 10^{−6}), one SNP in 14q12 (rs1956217, *P* = 7.9 × 10^{−6}), one SNP in 17q11.2 (rs4239268, *P* = 9.9 × 10^{−6}), and three SNPs in 19q13.1 (the best SNP: rs10500279, *P* = 9.5 × 10^{−7}). The signal plots for each of the eight loci are shown in Supplementary material online, Figures S2–S9.

Validation in replication sample

Twelve of the 14 SNPs that we identified were genotyped in the replication sample (207 cases and 597 controls). All genotypes had low missing rate (0.1–3.2%) and none of SNPs failed to meet the criteria of HWE. Of the 12 SNPs, 3 SNPs in *RYR1*, ryanodine receptor 1 (skeletal) in 19q13.1 were replicated at *P*-values that ranged between 2.7 × 10^{−2} and 3.6 × 10^{−2} in the hospital-based sample (Figure 2 and Table 2). Moreover, the combined analysis of the KARE/replication sample demonstrated that the association signals of all three SNPs passed the threshold of significance for genomewide associations (*P* < 7.2 × 10^{−8}).²⁶ The three SNPs lay in introns of *RYR1* gene, and rs10500279, the most significant SNP, had an odds ratio (OR) of 1.58 [confidence interval (CI): 1.35–1.85] and *P* = 1.0 × 10^{−8} (Figure 2 and Table 2).

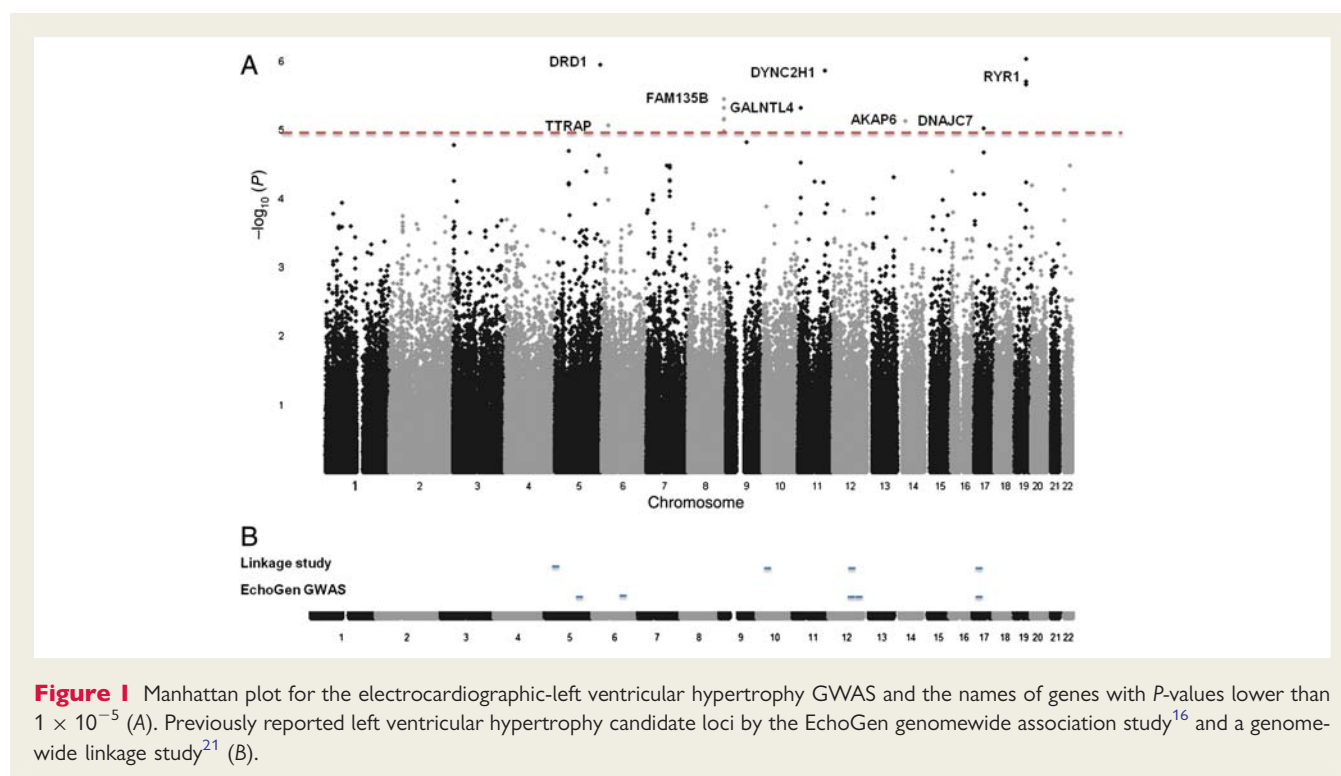


Figure 1 Manhattan plot for the electrocardiographic-left ventricular hypertrophy GWAS and the names of genes with P -values lower than 1×10^{-5} (A). Previously reported left ventricular hypertrophy candidate loci by the EchoGen genomewide association study¹⁶ and a genomewide linkage study²¹ (B).

Discussion

In this GWAS of ECG-LVH using community-based cohorts, three SNPs in *RYR1* in the 19q13.1 locus approached significance; these were replicated in a hospital-based cohort. This GWAS of ECG-LVH contributes to the existing body of literature, which, until now, has focused on ECHO-LVH; studies on the ECG indices reflective of LVH may reflect disparate biological processes compared with anatomical measures by echocardiography.⁷

A report on the genomewide linkage of ECG-LVH by Mayosi *et al.*²¹ identified three suggestive loci: 10q23 for Sokolow–Lyon voltage, 17p13.3 for Cornell voltage product, and 12q14.1 for ECG LV mass. As shown in Figure 1B, no loci overlapped between this report and our findings, which might be attributed to disparate methods of establishing the LVH criteria (we used the Minnesota Code Classification System)²³ and differences in the power of detection between linkage studies and GWASs.²⁷ In addition, it was difficult to identify LVH-associated loci that overlapped between the EchoGen GWAS¹⁶ and this study, which might reflect the disparate biologies of ECG-LVH and ECHO-LVH.

Several limitations should be addressed with regard to the interpretation of our results. In this study, only Minnesota ECG criteria were used to diagnose ECG-LVH cases. However, Hsieh *et al.*²⁸ suggested that compared with voltage-only criteria for detecting LVH, composite ECG criteria were more strongly predictive of CV mortality. Further, ECG voltage criteria detect LV wall thickness indirectly. Second, because the sample size of the replication study was not large, we could not rule out the possibility that other suggestive loci, such as *DRD1*, a known CV risk factor, are associated with ECG-LVH.²⁹ To confirm

other suggestive loci, they must be examined in a larger association study.

RYR1 has been well studied with regard to muscle contraction. Regulated increases in intracellular Ca^{2+} are required for many physiological functions, including muscle contraction, secretion, regulation of gene expression, and fertilization.³⁰ Intracellular Ca^{2+} can be elevated via activation of plasma membrane-bound Ca^{2+} -permeable channels or the release of Ca^{2+} from intracellular stores.³¹ The rise in Ca^{2+} that is required for myocyte contraction is affected by the activation of RYRs, sarcoplasmic reticulum-bound Ca^{2+} release channels.³²

Over the past 20 years, several mutations in *RYR1* and *RYR2* have been identified and linked to skeletal and cardiac diseases.³³ Malignant hyperthermia,³⁴ central core disease,³⁴ and catecholaminergic polymorphic ventricular tachycardia^{35,36} have been associated genetically with mutations in *RYR1* or *RYR2*. Further, several reports in animal models have demonstrated the involvement of RYRs in alterations in LVH, which observed that the density of RYRs decreased in hypertrophied rat hearts, resulting in impaired calcium flux,³⁷ and that RYRs were distributed transmurally during LVH in dogs.³⁸

Based on previous functional studies and the results observed in this study, it would be reasonable to speculate that RYRs are associated with LVH. It is difficult, however, to understand why *RYR1*, the predominant isoform in the skeletal muscle, has a stronger association with LVH than *RYR2*, the major isoform in the cardiac muscle [best SNP: rs3766884, OR = 0.81 (CI: 0.69–0.94), $P = 0.006$]. It is possible that *RYR1* or its genetic variants have stronger influence on LVH compared with that of *RYR2*. Otherwise, *RYR1* expression in the vascular smooth muscle

Table 2 Logistic regression analysis results of the original genomewide association study and replication study, controlling for antihypertensive drug-treated states, cohort, age, sex, and cardiovascular risk factors^a as covariates

Chr	rsID	Position	Locus	Proximal gene	M	KARE GWAS (cases 398/controls 8432)					Replication study (cases 207/controls 597)					Combined analysis (cases 605/controls 9029)			
						MAF	OR	L95	U95	P-value	MAF	OR	L95	U95	P-value	OR	L95	U95	P-value
5	rs265992	174846655	5q35.1	DRD1	A	0.1	1.70	1.37	2.10	1.2E−06	0.088	0.76	0.39	1.48	4.2E−01	1.46	1.20	1.78	1.6E−04
6	rs9295629	24654266	6p22.3-22.1	TTRAP	T	0.039	1.99	1.47	2.71	9.0E−06	0.037	0.61	0.17	2.13	4.4E−01	1.62	1.21	2.18	1.2E−03
8	rs6577840	138805432	8q24.2	FAM135B	C	0.159	1.52	1.27	1.83	7.1E−06	0.153	0.94	0.59	1.51	8.1E−01	1.38	1.18	1.63	9.2E−05
8	rs4909705	138805788	8q24.2	FAM135B	T	0.16	1.54	1.28	1.85	3.7E−06	0.149	1.00	1.00	1.01	7.7E−01	1.42	1.20	1.67	2.6E−05
8	rs7825068	138807660	8q24.2	FAM135B	T	0.159	1.53	1.28	1.84	5.0E−06	0.154	0.94	0.58	1.50	7.8E−01	1.39	1.18	1.63	8.0E−05
8	rs7840530	138811480	8q24.2	FAM135B	A	0.162	1.51	1.26	1.81	7.3E−06	0.153	0.93	0.58	1.51	7.8E−01	1.38	1.17	1.62	9.6E−05
11	rs17446021	11761070	11p15	GALNTL4	A	0.014	2.81	1.80	4.37	4.9E−06	0.011	5.63	0.74	42.88	9.5E−02	2.26	1.47	3.48	2.2E−04
11	rs11225822	103345030	11q21-q22.1	DYNC2H1	C	0.087	1.71	1.38	2.13	1.4E−06	0.091	1.35	0.74	2.48	3.3E−01	1.62	1.33	1.97	1.2E−06
17	rs4239268	40154777	17q11.2	DNAJC7	C	0.15	1.52	1.26	1.82	9.9E−06	0.131	0.86	0.51	1.46	5.8E−01	1.38	1.17	1.63	1.5E−04
19	rs2071090	39015454	19q13.1	RYR1	G	0.219	1.49	1.26	1.76	2.2E−06	0.236	1.59	1.05	2.41	2.7E−02	1.49	1.30	1.72	3.6E−08
19	rs10500279	39035068	19q13.1	RYR1	G	0.157	1.57	1.31	1.88	9.5E−07	0.173	1.65	1.03	2.65	3.6E−02	1.58	1.35	1.85	1.0E−08
19	rs2960321	39048163	19q13.1	RYR1	A	0.212	1.49	1.27	1.76	2.0E−06	0.232	1.59	1.04	2.42	3.1E−02	1.50	1.30	1.73	2.5E−08

Chr, chromosome; rsID, SNP ID in dbSNP database; BP, base position, based on the latest genome version (hg19); MAF, minor allele frequency; M, minor allele; OR, odds ratio; L95 and U95, confidence interval lower and upper 95%, respectively.

^aThe cardiovascular risk factors are BMI, SBP, DBP, HDL, LDL, TG, and fasting glucose level.

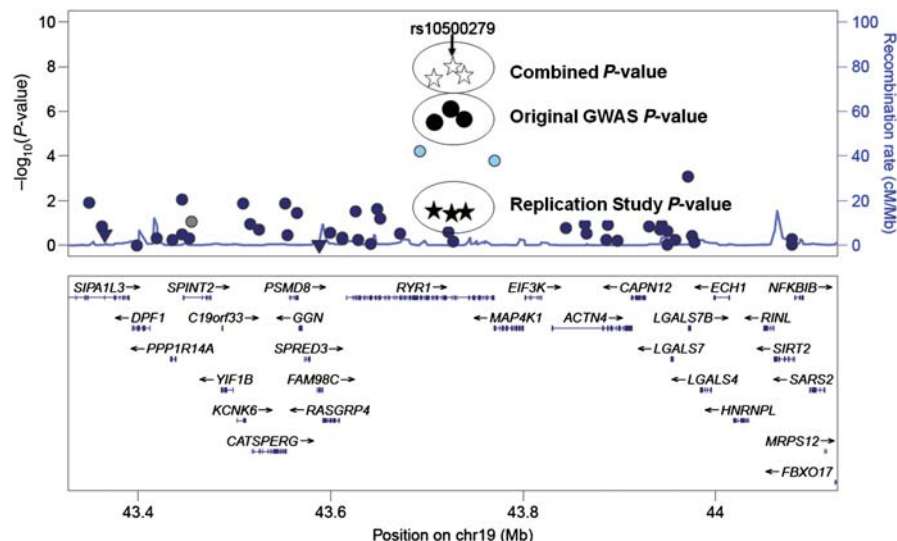


Figure 2 Signal plot for the *RYR1* locus in the genomewide association study (closed circle), replication study signals (closed stars), and combined study (open stars). Open circle indicates SNPs showing high pair-wise linkage disequilibrium ($r^2 > 0.8$), and closed triangle indicates nonsynonymous SNPs. The three single-nucleotide polymorphisms genotyped in the replication study are circled.

(rather than cardiac muscle) might regulate BP and affect ECG-LVH secondarily. To examine this possibility, we performed an association analysis of *RYR1* SNPs with SBP and DBP. However, there was no association found in this analysis, indicating that this possibility is low (data not shown).

Therefore, to better understand the mechanism responsible for the potential association between *RYR1* and ECG-LVH, further examination through functional analysis of *RYRs* is necessary.

In conclusion, we have reported the first GWAS of ECG-LVH, with which variations in *RYR1* were associated and this finding will increase our understanding of the aetiology of ECG-LVH.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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Conflict of interest: none declared.

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